REMARKS

Claims 1, 2, 4-9, 13-27, 29-43, 45, and 46-49 were pending prior to this response. By the present communication, no new claims have been added, claim 49 has been cancelled and claims 17, 39, 46 and 47 have been amended to define the invention with greater particularity. Accordingly, claims 17, 19, 24, 25, 29, 30-32, 34, 39-43, and 45-48 are currently pending.

The amendments add no new matter, being fully supported by the Specification and original claims. In particular, the amendment to claims 17, 39 and 47 has been made to further clarify the metes and bounds of the claims by requiring that bone marrow early attaching cells are not obtained from the peripheral blood circulation, but are obtained by culturing of bone marrow itself, which is the crux of the invention. Support for the amendment is found throughout the Specification, for example at paragraph [0033], lines 1-5; paragraph [0044], line 1, and paragraph [0100].

The amendment to claim 46 is made to adjust the claim language to that more suitable for a method claim by specifying the processes involved in preparing the composition from aspirated bone marrow.

The Rejection under 35 U.S.C. § 103(a)

A. Applicants respectfully traverse the rejection of claims 17, 19, 24, 25, 35, 39-42 and 45-48 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al.et al (Angiogenesis and Vasculogenesis, Heart, Urban and Vogel, Vol. 25 No. 6 (2002) pages 611--622 (hereinafter "Kalka et al.et al.") in combination with Chiu et al. U. S. printed publication 2002/0197240 (hereinafter "Chiu"), claim 49 having been cancelled and claims 17 and 48 having been amended. Applicants disagree with the Examiner's assertion that the subject matter of claim 17, as presented, would be obvious to those of skill in the art under 35 U.S.C. § 103(a) over Kalka et al.in view of the disclosure of Chiu. Applicants submit that the subject matter of claim 17 distinguishes over the combined disclosures of Kalka et al.and Chiu by reciting:

"A method for enhancing collateral blood vessel formation in heart or limb muscle tissue, said method comprising:

directly injecting into a site of impaired blood flow in heart or limb muscle tissue an effective amount of early attaching cells obtained from autologous bone marrow, which early attaching cells have been transfected in vitro with an adenoviral vector comprising a polynucleotide encoding one or more angiogenic factors selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS)."

Applicants respectfully submit that Kalka et al.neither teaches nor suggests the use of early attaching cells obtained by culturing bone marrow as a gene therapy expression cell for delivering to ischemic muscle tissue one or more angiogenic agents that enhance development of collateral blood supply, wherein the angiogenic agent(s) are expressed by the early attaching cells in vitro. However, the combined teaching of Kalka et al.and Chiu fail to disclose or suggest the claimed invention under 35 U.S.C. § 102 or 103(a). Applicants are the first to invent a method whereby the step of extracting EPCs from peripheral blood can be eliminated and replaced by culturing bone marrow cells to obtain therefrom early attaching cells for injection into ischemic muscle issue in heart or limb to induce development of collateral blood vessels. Moreover, Applicants are the first to disclose that cells obtained from bone marrow by culturing of such cells can be transfected by and will express in vitro DNA encoding a therapeutic angiogenesis factor to produce conditioned medium containing therapeutic angiogenesis factors.

The Examiner asserts that those of skill in the art would be motivated to improve upon Kalka's disclosed methods because of the known paucity of EPCs in peripheral circulation. However, the Examiner fails to document that the initial population of EPCs in the early attaching cells obtained by culturing bone marrow was known by those of skill in the art to be substantially greater than that can be extracted from peripheral blood circulation. Moreover, Applicants submit that it was not know in the art nor does the Examiner provide evidence showing that bone marrow cells extracted from peripheral circulation are substantially "the same" or would suggest use of those obtained by culturing bone marrow. In fact, Applicants submit that it was well known in the art PECs, such as those disclosed by Kalka, are influenced in their development by the in vivo environment in which they are

found (See for example, Chiu et al. of record herein). In addition, the bone marrow cells used in the experiments described by Kalka et al.are not identified as autologous to the patient treated. Therefore, Applicants respectfully submit that the Examiner fails to show that the bone marrow cells used by Kalka et al.are the same or would function the same in vivo as the early attaching cells obtained by culturing bone marrow.

In addition, Applicants respectfully submit that Kalka et al.fail to suggest that bone marrow cells obtained from bone marrow aspirate of the subject to be treated can be cultured in vitro to obtain an expanded population of early attaching cells compatible with the subject so that no immune reaction will result from injection of such cells or that such cells can be successfully (i.e, at a suitable transfection ratio) transfected by one or more adenoviral vectors encoding the angiogenic factors and will express such angiogenic factors as are specified in claim 17, either in vitro or in vivo. In fact, Applicants submit that Kalka et al. "teach away" from the invention by recommending expansion of the population of circulating EPCs by prior injection of VEGF (or free plasmid DNA encoding a therapeutic angiogenic factor) into peripheral blood of the donor.

In addition, Applicants submit that Kalka et al. are absolutely silent regarding "further culturing" of such transfected early attaching cells so as to express into conditioned medium one or more of the said therapeutic angiogenic factors to obtain a composition that can be additionally injected as an adjunct to the transfected early attaching cells to enhance development of collateral blood supply in ischemic muscle tissue in heart or peripheral limb.

With regard to "therapeutic angiogenesis", Kalka et al. fail to enable transfection of and expression of any angiogenic factor by EPCs, whether in vitro or in vivo because Kalka describe only expression of a marker protein by transfected EPCs. Moreover, although Kalka et al. may suggest the possibility of "postnatal neovascularization" in adults, Kalka et al. issue the following warning: Moreover, Kalka's suggestion, if any such be, is accompanied by a warning that stimulating postnatal neovascularization may also "stimulate a pathological neovascularization" (Kalka et al., page 27).

"Further research must … be carried out …with regard to a possible therapeutic use of endothelial progenitor cells within the scope of a cell therapy for the regeneration of ischemic tissue" (Kalka et al.,

page 28). In view of these warnings, Applicants submit that the reference would, at most, only motivate those of skill in the art "to try" administration of recombinant EPCs of any type for adult angiogenesis, such as regeneration of ischemic tissue, much less use of early attaching cells obtained by culturing of bone marrow as expression cells for producing one or more stimulatory transgenic angiogenic factors in vitro to stimulate the cascade of endogenous angiogenic factors blood marrow-derived cells are known to produce when implanted in ischemic tissue.

Furthermore, even if those of skill in the art were motivated by the disclosure of Kalka et al to find a new source of EPCs for use in "therapeutic angiogenesis", Applicants submit that Kalka's speculation about the danger of "therapeutic angiogenesis" would fail to provide those of skill in the art with expectation of success necessary to constitute obviousness under the statute.

Furthermore, with respect to the invention of claim 46, there is no evidence provided in the cited art or knowledge of the art as referenced by the Examiner that cells obtained from cultured bone marrow aspirate, if transfected with one or more adenoviral vector(s) encoding one or more stimulatory angiogenic factor(s), could be used to prepare conditioned medium containing transgenically expressed angiogenic factors for use as an adjunct to angiogenic cell therapy.

With regard to whether Kalka et al. suggest using transfected PECs in cell therapy, Applicants submit that the reference discloses only "the possibility of utilizing" transfected EPCs (obtained from peripheral circulation) for formation of vessels by over-expression of VEGF (Kalka et al, page 27). Actual transfection of EPCs disclosed in Kalka et al. pertains to expression of β-galactosidase in cells of the endothelium cell line in the bone marrow of a mouse (Kalka et al., pp. 18-19). Again, Applicants submit that Kalka et al, at best, would motivate those of skill in the art only to "try" injection into ischemic tissue of transfected EPCs obtained from peripheral circulation for secretion in vivo of a stimulatory angiogenic factor.

The Examiner, in fact, admits that "Kalka et al. do not teach a method for enhancing collateral blood vessel formation by using early attaching cells obtained from bone marrow transfected with an adenoviral vector encoding one or more of the angiogenic factors such as HIF-1, EPAS1, MCP-1 GM-CSF, etc. (Office Action, page 3). To overcome the differences between Kalka et al. and the claimed subject matter, the Examiner relies upon Chiu et al. However, disclosure of Chiu et al. pertains

primarily to myogenesis, not to angiogenesis. Chiu et al. disclose injection into an MI patient of autologous marrow stromal cells that have been modified to express a cardiomyocyte phenotype in vitro. A second "non-modified" group of marrow stromal cells is disclosed by Chiu et al. as functioning to "differentiate into angiogenesis" to provide the necessary blood flow for development of the modified implanted cells (Chiu [0160] and [0161]). Thus, the therapeutic goal of Chiu et al. is myogenesis, not angiogenesis. This is different than the therapeutic goal of the present claims, which is to develop collateral blood vessels in the vicinity of the ischemic area. Moreover, Chiu et al. specify that the cells that lead to angiogenesis are to be "non-modified", a description that Applicants submit would actually discourage transfection of such cells to achieve angiogenesis.

Despite these differences between the claimed subject matter and the combined disclosures of Chiu et al and Kalka, et al., the Examiner asserts that their combined disclosures would suggest the implantation of transfected (i.e. "modified) marrow stromal cells to cause development of collateral blood flow.

In addition, Applicants submit that Chiu et al. are absolutely silent regarding use of transfected cells derived from culture of bone marrow cells to enhance collateral blood flow. Chiu et al. refer to use of "labeled cardiac myocytes and fibers" (Chiu et al, paragraph [0037]], to track incorporation of myocytes into ischemic or damaged heart muscle, but do not contemplate transfection of MSCs with any therapeutic angiogenic factor, such as one that will be administered to a subject in conditioned medium into which it has been expressed in vitro. Instead, as in the Kalka reference, Chiu et al. stress that therapeutic use of implanted MSCs is in the experimental stage:

> The role of cytokines and other growth factors will . . . be examined in the future. Further still, methodologies for transplanting autologous MSCs in patients to improve cardiac function will be optimized in future studies.

(Chiu et al., paragraph [0040]. In view of the reservations and uncertainties disclosed by both pieces of art cited, Applicants respectfully submit that the combined disclosures of Kalka et al. and Chiu et al. would, at the most, motivate those of skill in the art "to try" the invention as defined by the claims at issue here, but would not provide those of skill in the art with an expectation of success.

Consequently, Applicants respectfully submit that the differences between the cited art and the present invention, as currently recited in claims 17 and 39, are such that the subject matter as a whole would not have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 17, 19, 24, 25, 34, 39-42 and 45-48 under 35 U.S.C. §103(a) as being unpatentable over the combination of Kalka et al. and Chiu et al.

B. Applicants respectfully traverse the rejection of claims 29-32 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al. and Chiu et al as applied above and further in view of Hamawy et al. Smith et al. and Li et al. Applicants' remarks above regarding the differences between the disclosures of Kalka and Chiu and the invention methods for enhancing collateral blood vessel formation applies equally to the rejection of claims 29-32 and is incorporated here by reference.

In addition Applicants disagree with the Examiner's reliance upon Hamawy, et al., Smith et al. and Li et al. as providing the suggestion, teaching or motivation needed for those of skill in the art to overcome the differences between the combined disclosures of Kalka et al.and Chiu such that the subject matter of claims 29-32 would have been obvious under 35 U.S.C. § 103(a) to those of skill in the art at the time of the invention.

The Examiner alleges, for example, that Hamawy et al. identify over 20 angiogenic factors associated with revascularization of ischemic muscle tissue, including the VEGF disclosed by Kalka et al. and the (FGF)s as recited in claim 39 (Office Action, page 5). In summary, the Examiner states: "Further, given the level of skill in the art at the time of invention there would have been a reasonable expectation of success in replacing one angiogenic factor with another" (Office Action, page 6). However, the Examiner fails to point out any passages in Hamawy, et al., Smith et al. and Li et al., other than those disclosing yet another angiogenic factor endogenously produced by an individual suffering from ischemic muscle tissue, that would overcome the differences between the combined disclosures of the primary references (as discussed above and

incorporated here) and the subject matter of claims 29-32, which contain all the requirements of claim 17. Accordingly, Applicants submit that the cited art fails to establish prima facie obviousness of the subject matter of claims 29-32 under 35 U.S.C. § 103(a) and reconsideration and withdrawal of the rejection are respectfully requested.

C. Applicants respectfully traverse the rejection of claim 43 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al.and Chiu et al as above and further in view of Tomika. With regard to the primary references cited, Applicants' remarks above regarding the differences between the invention methods for enhancing collateral blood vessel formation and the disclosures of Kalka and Chiu apply equally to the rejection of claim 43, which incorporates all the limitations of claim 17, and are incorporated here by reference.

The Examiner relies upon Tomika as disclosing that when obtaining bone marrow derived cells it is beneficial to have an anticoagulant present and alleges with respect to claim 43: ". . . it would have been obvious to add heparin to the aspirate to obtain the benefit of preventing clotting or coagulation of a composition comprising bone marrow, which in turn comprises cells that are expanded/transfected *ex vivo.*" (Office Action, page 7)

However, the Examiner fails to point out any passages in Tomika, other than those referring to the utility of including an anticoagulant in a composition comprising bone marrow cells, that pertain to the present claims. In particular the Examiner fails to point out how Tomika would supplement and overcome the differences between the disclosures of the primary references cited and the subject matter of claim 43, which depends from and includes all the limitations of claim 1. Accordingly, Applicants submit that the cited art fails to establish prima facie obviousness of claim 43 under 35 U.S.C. §103 and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph.

Applicants respectfully traverse the rejection of claims 45 and 46 under 35 U.S.C. § 112, second paragraph for being indefinite as allegedly failing to point out and claim the subject matter of the invention.

With regard to the word "derived" in these claims, the Examiner asserts that the nature and number of derivative processes are unknown so that those of skill in the art would be uncertain of the metes and bounds of the claims. To overcome the grounds of the rejection Applicants have amended claims 45 and 46 to delete the phrase "derived from bone marrow" and to substitute in its place the phrase "obtained by culturing bone marrow". Thus, the amendment clarifies that the early attaching cells are not obtained from the peripheral circulation of a subject, but instead are obtained by culturing of the bone marrow of a subject.

Accordingly Applicants submit that claims 45 and 46 as currently amended meet all requirements under 35 U.S.C. § 112, second paragraph. Reconsideration and withdrawal of the rejection of claims 45 and 46 as being indefinite, therefore, are respectfully requested.

The Commissioner is hereby authorized to charge the amount of $510.00 as payment for a Three Month Petition for Extension of Time fee. No other fee is deemed necessary with the filing of this paper. However, the Commissioner is hereby authorized to charge any other fees that may be required by this paper or credit any overpayments to Deposit Account No. 07-1896, referencing the above-identified docket number.

Respectfully submitted,

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